

EFFECT OF ENZYME CONCENTRATION AND TEMPERATURE
ON VISCOSITY AND BETACYANIN CONTENT FROM PITAYA
WASTE EXTRACT

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NOMENCLATURE

°C	Degree Celsius
%	Percentage
MPa	Mega Pascal
USD	United States Dollar
RM	Ringgit Malaysia
Tons	Tonnes
\$	Dollar
pH	Power of Hydrogen
°F	Degree Fahrenheit
g/cm ³	Gram per centimeter cube
µm	Micro meter
mm	Millimeter
M	Molarity
V	Volume
mL	Milli-Liter
g	Gram
h	Hour
cP	Centipoises
wt.	Weight
s	Second

KESAN KEPEKATAN ENZIM DAN SUHU TERHADAP KELIKATAN DAN KANDUNGAN BETASIANIN DARIPADA EKSTRAK KULIT BUAH PITAYA

ABSTRAK

Oleh kerana pencelup buatan menunjukkan kesan negatif dalam bidang makanan dan tekstil, pewarna semulajadi telah mendapat keutamaan terutamanya dalam bidang tekstil. Kulit buah naga digunakan sebagai sumber untuk pewarna semulajadi dalam eksperimen ini. Terdapat satu kekangan yang menghalang pewarna semulajadi yang dihasilkan untuk melekat pada kain kerana sifat fizikalnya yang sangat likat yang mana dipercayai disebabkan oleh kandungan pektin. Kajian ini bertujuan untuk mengurangkan kelikatan pewarna semulajadi dengan menggunakan enzim yang bernama pektinase dan dijalankan dalam skala yang kecil. Eksperimen ini juga bertujuan untuk mengkaji kesan kepekatan enzim dan suhu terhadap pengurangan kelikatan dan kandungan pigmen betasianin dalam pewarna semulajadi. Eksperimen ini menggunakan kaedah kimia dan biologi dimana kaedah kimia merujuk kepada pengekstrakan pewarna semulajadi daripada kulit pitaya menggunakan air sebagai pelarut. Manakala, kaedah biologi pula merujuk kepada tindakbalas enzim yang digunakan untuk mengkurangkan kelikatan pewarna semulajadi. Dua faktor yang dikaji dalam eksperimen ini iaitu kesan kepekatan enzim dan suhu telah menunjukkan kepekatan enzim dan suhu yang optimum terhadap pengurangan kelikatan pewarna semulajadi. Pada kepekatan enzim 2.5% dan suhu 50°C, pengurangan kelikatan yang amat besar dan pengurangan pigmen betasianin yang sedikit dalam pewarna semulajadi dapat diperhatikan. Daripada kajian ini, adalah dicadangkan bahawa ujikaji lanjutan boleh dilaksanakan terhadap pH dan kepekatan pewarna semulajadi bagi mendapatkan kelikatan yang rendah dan kandungan betasianin yang tinggi.

EFFECT OF ENZYME CONCENTRATION AND TEMPERATURE ON VISCOSITY AND BETACYANIN CONTENT FROM PITAYA WASTE EXTRACT

ABSTRACT

As synthetic dye has shown up few hazards in contributing in food and textile industries, natural dye has gain its priority in those fields especially in textile sector. In this experiment, pitaya's waste was selected as a source for natural dye. Thus, there is one obstacle that prevents the natural dye to fulfill the requirements needed in textile industries as the physical properties of the natural dye which is high in viscosity causing it not fasting on cloth which is believed, due to pectin content. In order to come over this problem, this research aim to reduce the viscosity of the natural dye using commercialize pectinase, in a small scale and study the effect of the enzyme concentration and temperature on the reduction of viscosity of the natural dye and also observe the difference in betacyanin content. This experiment was carried out by chemical and also by biological mechanism. Chemical mechanism refers to solvent extraction using water to extract the dye from the fruit, whereas, biological manner refers to usage of enzyme to reduce the viscosity of the natural dye. When enzyme concentration varies form 0.1 % to 5%, the viscosity reduced gradually until the enzyme concentration is 2.5% then the reduction is insignificant. Whereas, the temperature shown a similar result. The highest reduction in viscosity is when the reaction temperature is set at 50°C. It is because, when temperature increases, the rate of reaction will increase and at one point, the rate of reaction will decrease. It is because the enzymes will be denatured at high temperatures. From this research, it is recommended that further studies can be done to the pH and concentration of natural dye in order to obtain low viscosity and high betacyanin content.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Colours play an important role in enhancing the aesthetic appeal for food and clothing products. In ancient times, natural dye which is obtained from plants pigment through biological manner was commercialized. Then, as technology and exploration in chemical industries enhanced, synthetic dye was introduced and undeniable it gained a positive response from food and textile manufacturer as it is cheap, easier to apply, more colorfast, and could be produced in a wider and brighter range of colors. Slowly, when hazards of synthetic dye come to the public attention, its position as a dye is threatened. Again, natural dye has gained its popularity back due to its nature characteristics.

As a consequence, extraction colour from plant has gained much attention from many researches. Precisely, colour properties from pitaya waste can be alternative way to replace synthetic chemical in many fields of industries. Production of natural dye from pitaya waste can be optimized since there is a growing interest in

the use of natural pigments for food colouring because natural products are associated with quality and health promotion whereas synthetic pigments are critically assessed by consumers (Downham and Collins, 2000).

1.2 Problem Statement

In order to reduce the usage of synthetic chemical as a conventional dye, there are alternative ways by using natural resources such as pitaya waste to produce natural dye. It is undeniable that pitaya fruit has all the creditability to produce a natural dye, there are still crisis encountered in the application of the dye obtained from the pitaya waste. Hence, using waste to transform to beneficial product will reduce many environmental issues. Other than that, natural dye will be more safer and environmental friendly to use in textile industries. Although the natural dye has all its advantages, the problem occurred when the dyes are harder applied onto the fabrics. Concisely, the colour not fasting on the fabric and it is easily removed from the fabric. It has been determined that the physical property of the dye such as high viscosity, resulting in the mentioned scenario. Due to its features, it is deduced that one of the possible component that caused the dye to show up such characteristic is pectin.

1.3 Research Objectives

This study is carried out:

- i) To investigate the effect of enzyme concentration and temperature on viscosity of the natural dye.
- ii) To investigate the effect of enzyme concentration and temperature on betacyanin content of the natural dye.
- iii) To compare the fastness of the natural dye before and after the enzymatic reaction.

1.4 Scope of the Study

The scopes of study are:

- i. Red pitaya waste is used which was bought in Taman Tas.
- ii. The dye extracted from the fruit using water extraction by applying ratio 1:3 of 1g of raw material to 3 mL of water.
- iii. Sedimentation through centrifugation is carried out for half an hour under 4°C at 8000 revolution per minute (rpm) and filtration through stainless steel filter fabric (0.3 mm mesh) was used to separate the insoluble residue (Harivaindaran et al., 2008).
- iv. Pectinase enzyme was used to reduce the viscosity of the natural dye in pH of 5.5 and at 50°C.

- v. The effect of enzyme concentration (0.1 % to 5%) and temperature (30°C to 100°C) on reduction of viscosity and changes in betacyanin content will be studied.

1.5 Significance of the Study

This study is conducted in order to reduce the viscosity from the dye extracted from pitaya peel. The reduction in viscosity is extremely beneficial for the dye as it increases the dye's properties in term of stability. This accent will make the dye to last long onto the fabrics and suitable used as a conventional dye in textile industries.

In this study, commercialize pectinase enzyme will be used to break down the pectin content which is believed to cause the high viscosity in the dye. The effect of enzyme concentration and temperature will be studied on reduction of viscosity and betacyanin content in the natural dye. The finding from this study hopefully will be useful for the industrial especially textile industries and economic purpose.

CHAPTER 2

LITERATURE REVIEW

2.1 Natural Dye

Natural dyes are dyes or colorants derived from plants, invertebrates, or minerals. The majority of natural dyes are vegetable dyes from plant sources – roots, berries, bark, leaves, wood and other organic sources such as fungi and lichens. The earliest surviving evidence of textile dyeing was found at the large Neolithic settlement at Çatalhöyük in southern Anatolia, where traces of red dyes, possible from ochre (iron oxide pigments from clay), were found. Polychrome or multicolored fabrics seem to have been developed in the 3rd or 2nd millennium BCE (Barber, 1991). Textiles with a "red-brown warp and an ochre-yellow weft" were discovered in Egyptian pyramids of the Sixth Dynasty (2345-2180 BCE). There is a growing interest in the use of natural pigments for textile coloring because natural products are associated with quality and health promotion whereas synthetic pigments are critically assessed by consumers (Downham and Collins, 2000). Natural colorants from plant sources are receiving growing interest from both textile manufacturers and consumers in the continuing replacement of synthetic dyes (Duhard et al., 1997;

Stintzing and Carle, 2004). In the early 21st century, the market for natural dyes in the fashion industry is experiencing a resurgence (Calderin and Jay, 2009) Western consumers have become more concerned about the health and environmental impact of synthetic dyes in manufacturing and there is a growing demand for products that use natural dyes. The European Union, for example, has encouraged Indonesian batik cloth producers to switch to natural dyes to improve their export market in Europe.

2.1.1 Classification of Natural Dye

Natural dyes can be classified (Gulrajani and Gupta, 1992) in a number of ways. The earliest classification was according to alphabetical order or according to the botanical names. Later, it was classified in various ways, e.g. on the basis of hue, chemical constitution, application class etc. In the colour index the natural dyes are classified according to the hue (Predominating colour). The number of dyes in each hue is as follows in table 2.1:

Table 2.1 The number of natural dyes in each hue as per the colour index.(Source: Gulrajani and Gupta, 1992)

CI Natural	No. of Dyes	Percent
Yellow	28	30.4
Orange	6	6.5
Red	32	34.8
Blue	3	3.3
Green	5	5.5
Brown	12	13
Black	6	6.5

On the basis hues, natural dyes can be classified as follows (Dedhia, 1998)

- i) Red colour dyes: most red dyes are hidden in roots or barks of plants or camouflaged in the bodies of dull grey insects. They are almost invariably based on anthraquinone and its derivatives. These dyes are stable to light and washing.
- ii) Yellow colour dyes: Yellow is the liveliest and perhaps the most abundant of all hues in nature. About 90% of the yellow dyes are flavonoids. Generally, they produce pale shade with quicker fading except turmeric, which produce dull deep shade but considered to be susceptible to light as they emit fluorescence. Wash fastness rating of natural yellow dyes ranges from fair to excellent, e.g., tesu, turmeric, kapila.
- iii) Blue colour dyes are indigo and woad, give excellent fastness to light and washing.
- iv) Black colour dyes: Black shades, generally obtained from tannin rich plant natural dyes and appreciably substantive towards cellulosic and protein fibre, imparts good overall fastness properties. Examples – logwood, harda, custard apple etc.

Natural dyes can also be classified on the basis of their chemical constitution (Dedhia,1998).

- i) Indigoid dyes: Indigo and tyrian purple are the most common examples of this class. Another blue dye, woad also possesses indigo as the main dyeing component.

- ii) Anthraquinone dyes: Almost all the red natural dyes are based on the anthraquinoid structure having both plant and mineral origin. Madder, lacs, kermes, cochineal are some of the dyes possess this type of structure. These are generally mordant dyes.
- iii) Alphanaphthoquinones: Typical example of this class is lawsone (henna), cultivated mainly in India and Egypt. Another similar dye is juglone, obtained from the shells of unripe walnuts. These dyes are generally disperse dyes and give shades of orange.
- iv) Flavonoids, which yield yellow dyes can be classified under flavones, isoflavones, aurones and chalcones. Flavones are colourless organic compounds. Most of the natural yellows are derivatives of hydroxyl and methoxy substituted flavones and isoflavones. Common example is weld (containing luteolin pigment) giving brilliant and fast colours on both wool and silk.
- v) Betalains actually comprised of two groups of pigments: the red–purple betacyanidins and the yellow betaxanthins both of which are water-soluble. Betacyanidins are conjugates of cyclo-DOPA and betalamic acid, and betaxanthins are conjugates of amino acids or amines and betalamic acid.
- vi) Anthocyanidins: The naturally occurring member of this class includes carajurin, a direct orange dye for wool and cotton. It is obtained from the leaves of bignonia chica.
- vii) Carotenoids: The class name carotene is derived from the orange pigment found in carrots. In these, the colour is due to the presence of long conjugated double bonds.

2.2 Source of Natural Dye

Natural dye are derived from naturally occurring sources such as plants (e.g., indigo and saffron); insects (e.g., cochineal beetles and lac scale insects); animals (e.g., some species of mollusks or shellfish); and minerals (e.g., ferrous sulfate, ochre, and clay) without any chemical treatment (Samantha et al., 2009).

2.2.1 Natural Dye from Animal

A good example is cochineal, which is a brilliant red dye produced from insects living on cactus plants. The properties of the cochineal bug were discovered by pre-Columbian Indians who would dry the females in the sun, and then ground the dried bodies to produce a rich, rich red powder (Vankar, 2000). When mixed with water, the powder produced a deep, vibrant red coloring. Cochineal is still harvested today on the Canary Islands. In fact, most cherries today are given their bright red appearance through the artificial color "carmine", which comes from the cochineal insect. Natural dyes are derived from animals are summarized below:

- i) Cochineal insect (Red)
- ii) Cow Urine (Indian Yellow)
- iii) Lac insect (Red, Violet)
- iv) Murex Snail (Purple)
- v) Octopus (Sepia Brown)

In this research, natural dye from animal source did not consider, because mostly, in order to extract the dye from animal, the animals has to be dead. So, if massive application of natural dye is extracted from animal or insects, it may harm the population of the indicated lives. Hence, the extraction of dye from animal requires a more tedious and involves chemical substances which may cause harm to the users.

2.2.2 Natural Dye from Plant

Many plants have been identified as potentially rich in natural dye contents, and some of them have been used for natural dyeing for quite some time. Various parts of plants like roots, stems barks, leaves, fruits and seeds may contain colouring matter which can be exploited. Normally, natural dyes are extracted from the roots, stems, leaves, flowers, fruits and vegetables (Vankar, 2000). Some plants may have more than one colour depending upon which part of the plant one uses. The shade of the colour a plant is picked, how it was grown, soil conditions, etc. the minerals in the water used in a dye bath can also alter the colour. Some natural dyes contain natural mordants. About 500 plant origin dyes, colouring matter derived from root, leaf, trunk, and others are shown in the Table 2.2 below.

Table 2.2 Common natural dyestuff obtained from different vegetable origin.
(Source Ashis and Adwaita, 2002)

Part of the plants	Dyestuff
Root	Turmeric, Madder (Manjistha), Onions, Beet-root
Bark/ Branches	Purple bark, Sappan wood, Shillicorai, Khair, Red, Sandalwood
Leaf	Indigo, Henna, Eucalyptus, Tea, Cardamon, Coral Jasmine, Lemon Grass
Flowers (Petals)	Marigold, Dahlia, Tesu, Kusum
Fruits/Seeds	Latkan, Pomegranate rind, Beetle nut, Myrobolan (Harda)

Natural dye can be extracted from plant easily without any chemical substances mostly, by water extraction. In addition, extraction of dye from plant can be extracted mostly from waste such as from peel or small portion of plant which will not give effect to the plant. In Malaysia, the society generates abundant agricultural wastes with the volume of approximately 5 million tones annually and is expected to double by the year 2010 (Park et al., 2007). Some of these wastes include oil palm trunks and fronds, palm kernel cake, sugar cane baggase, rice husk, rice straws, coconut fibers and meal, cocoa pods, rubber wood dusts, fruit peels and many other wastes materials.

The management of these wastes effectively and economically must be given utmost priority in the country in ensuring not only in reducing the detrimental impact of the wastes to the environment, but most importantly in the transformation of these wastes into useful raw materials for the production of added value commodities of industrially commercial potentials. Annually, roughly 2534.2 ton dragon fruits produced in Malaysia (Cheah and Zulkarnain 2008). The peels are mostly waste material resulting from the dragon fruit juice processing industry and normally discarded. These discarded peels, as mentioned above, may cause severe

problem especially to environment particularly water pollution. Since, pitaya peel has a high content in betacyanin pigment, it has all the creditability to be a sustainable source for natural dye production in Malaysia.

Betalains are a class of red and yellow indole-derived pigments found in plants of the Caryophyllales, where they replace anthocyanin pigments (Clement and Mabry, 1996). Betalains also occur in some higher order fungi. They are most often noticeable in the petals of flowers, but may color the fruits, leaves, stems, and roots of plants that contain them. They include powerful antioxidant pigments such as those found in beets. Betalains are the nitrogenous vacuolar pigments of 13 families within the plant kingdom also accumulating in some members of the Basidiomycetes (Gill and Steglich, 1987; Gill, 1994). The name "betalain" comes from the Latin name of the common beet (*Beta vulgaris*), from which betalains were first extracted. The deep red color of beets, bougainvillea, amaranth, and many cacti results from the presence of betalain pigments. The particular shades of red to purple are distinctive and unlike that of anthocyanin pigments found in most plants. There are two categories of betalains:

- Betacyanins include the reddish to violet betalain pigments.
- Betaxanthins are those betalain pigments which appear yellow to orange.

Among the betaxanthins present in plants include vulgaxanthin, miraxanthin and portulaxanthin, and indicaxanthin. Plant physiologists are uncertain of the function that betalains serve in those plants which possess them, but there is some preliminary

evidence that they may have fungicidal properties. The structure of betacyanin is shown in the Figure 2.1 below.

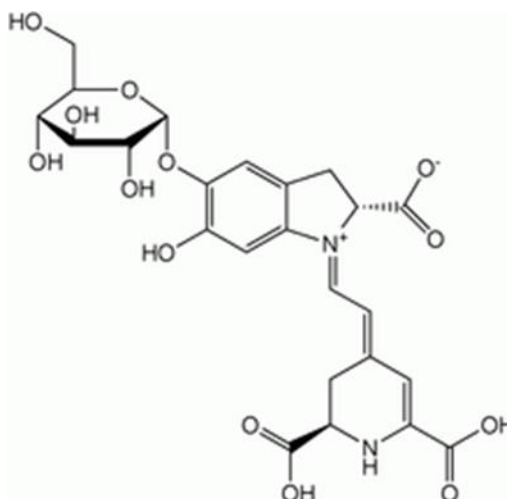


Figure 2.1 Betacyanin structure

Natural dye that contain betacyanin pigment was chosen to be extracted in this research because it exhibit a strong red colour which is has a strong attraction and made the studies to examine its property easier and clearer. Other than that, betacyanin pigment can be obtain in the products that easily available such as dragon fruit, and beet root. Hence, the betacyanin is also can be obtained from waste such as pitaya peel which has been selected in this research.